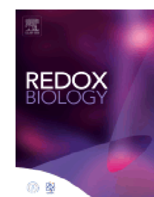
Redox Biology 8 (2016) 252–258

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox



Research Paper

Reduction of PCN biosynthesis by NO in *Pseudomonas aeruginosa*

Lei Gao [a,1], Yuying Zhang [b,1], Yan Wang [a], Xinhua Qiao [b], Jing Zi [a], Chang Chen [b,c,*], Yi Wan [a,**]

[a] Microbiology Institute of Shaanxi, 76 Xingying Road, Xi'an 710043, China
[b] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
[c] Beijing Institute for Brain Disorders, Beijing 100069, China

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form
16 October 2015
Accepted 19 October 2015
Available online 6 November 2015

Keywords:
Nitric oxide
PCN biosynthesis
Nitric oxide reductase
Pseudomonas aeruginosa
Cystic fibrosis

ABSTRACT

Pyocyanin (PCN), a virulence factor synthesized by *Pseudomonas aeruginosa*, plays an important role during clinical infections. There is no study of the effect of nitric oxide (NO) on PCN biosynthesis. Here, the effect of NO on PCN levels in *Pseudomonas aeruginosa* strain PAO1, a common reference strain, was tested. The results showed that the NO donor sodium nitroprusside (SNP) can significantly reduce PCN levels (82.5% reduction at 60 μM SNP). Furthermore, the effect of endogenous NO on PCN was tested by constructing PAO1 *nor* (NO reductase gene) knockout mutants. Compared to the wild-type strain, the Δ*nor* strain had a lower PCN (86% reduction in Δ*nor*). To examine whether the results were universal with other *P. aeruginosa* strains, we collected 4 clinical strains from a hospital, tested their PCN levels after SNP treatment, and obtained similar results, i.e., PCN biosynthesis was inhibited by NO. These results suggest that NO treatment may be a new strategy to inhibit PCN biosynthesis and could provide novel insights into eliminating *P. aeruginosa* virulence as a clinical goal.

© 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Pseudomonas aeruginosa is a gram-negative bacillus that is rapidly becoming one of the major causes of opportunistic and nosocomial infections. Nosocomial infections caused by *Pseudomonas aeruginosa* have become a worldwide problem. *P. aeruginosa* infections are associated with increased mortality and morbidity, particularly in susceptible patients with compromised immune systems and cystic fibrosis (CF); over 80% of CF patients die from these infections [1]. *P. aeruginosa* is naturally resistant to a large range of antibiotics (cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides) and may demonstrate additional resistance after unsuccessful treatment [2]. Some new strategies have been developed because of unsatisfactory traditional antibiotic treatment, including inhibition of the pathogenic factors of *P. aeruginosa* [3].

The main pathogenic factors of *P. aeruginosa* include elastase, alkaline protease, LasA protease, hemolysin, rhamnolipids and pyocyanin (5-methyl-1-hydroxyphenazine) (PCN) [4]. PCN, a blue colored phenazine exotoxin, can easily penetrate biological membranes and is found in the sputum of CF patients infected by *P. aeruginosa* [5]. Recent in vivo studies on alternative model hosts [6] and mice [7] have revealed that PCN is a key compound in *P. aeruginosa* infections and is a significant contributor to lung destruction during chronic *P. aeruginosa* infection in patients with bronchiectasis. PCN inhibits the ciliary beating of airway epithelial cells and enhances superoxide production [8].

Nitric oxide (NO) not only is important as a biological messenger but also has many biological effects [9]. Excessive exogenous NO can damage proteins, nucleic acids, and cellular membranes when the concentration of NO exceeds the capacity of cell metabolism. Nitric oxide reductase (NOR) is a common respiratory enzyme in eukaryotic cells and bacteria [10] that catalyzes the endogenous NO to N_2O. The enzyme is involved in the denitrification pathway of *P. aeruginosa* by dissimilatory nitrate respiration [11]. If NO is not reduced by NOR to N_2O, it may accumulate, and its toxicity could compromise bacteria. Therefore, the respiratory enzyme NOR is also a detoxifying enzyme. In other words, the enzyme is involved in the defense against exogenous NO in their surrounding natural habitats and within their hosts [12]. This enzyme is part of a cytochrome bc-type complex in *P. aeruginosa*. The *nor*C and *nor*B genes, encoding the cytochrome *c* and cytochrome *b* subunits, respectively, are clustered with the *nor*D gene, which is required for the expression of the active NOR enzyme [13].

Recent research has shown that a high concentration of NO can inhibit the growth of *E. coli* [14]; however, sublethal

* Corresponding author. Fax: +86 10 64871293.
** Corresponding author. Fax: +86 29 82357027.
E-mail addresses: changchen@moon.ibp.ac.cn (C. Chen), wanyi6565@sina.com (Y. Wan).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.redox.2015.10.005
2213-2317/© 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

concentrations of NO plays a great role in *P. aeruginosa* biofilm dispersal and helps in the biofilm mode of growth transition to the free-swimming planktonic state [15]. Is there any other effect of NO on *P. aeruginosa*? Based on this question, we tested the PCN biosynthesis of *P. aeruginosa* in the presence of different concentrations of the NO donor sodium nitroprusside (SNP). Both SNP and *nor* gene knockout mutants could effectively inhibit the synthesis of PCN in *P. aeruginosa.*

2. Material and methods

2.1. Bacterial isolates

Four clinical isolates of *P. aeruginosa* were collected from Shanxi Province People's Hospital in China (Table 1). The *P. aeruginosa* strains were isolated from urine (1) and sputum (3) samples. We also used the reference PAO1 strain as the main lab strain. The PAO1 strain was kindly provided by Professor Kangmin Duan (Department of Medical Microbiology, University of Manitoba, Canada).

2.2. Growth test

A 10 ml overnight culture of PAO1 was grown in LB medium (10% peptone, 5% yeast extract and 10% NaCl) at 37 °C on an orbital shaker at 200 rpm. The OD600 nm was adjusted to 0.1 using sterile LB and vortexed. Then, 100 μl was added to culture tubes containing 50 ml of LB medium. Next, sodium nitroprusside (SNP) was added to the LB in the tubes at the desired concentrations. For the untreated control, no SNP was added. The cultures were grown aerobically at room temperature on an orbital shaker at 200 rpm for 18–22 h. The growth was followed by measurement at OD600 nm every 2 h.

2.3. DNA manipulations

Chromosomal and plasmid DNA extraction, purification, enzymatic digestion, DNA ligation, and transformation of *Escherichia coli* were performed according to standard methods [16]. PCR was conducted using Taq polymerase (TaKaRa Japan) on an ABI PCR system. The following PCR program was used: 5 min at 95 °C and 30 cycles of 30 seconds at 95 °C, 30 seconds at annealing temperature (5 °C below the melting point), and 1 to 3.5 min (according to the size of the fragments) at 72 °C; 10 min at 72 °C; and then storage at 4 °C. The nucleotide and predicted amino acid sequences were analyzed using DNASTAR and compared with the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.4. Construction of P. aeruginosa PAO1 and PA515 nor gene knockout mutants

Protocols for DNA manipulation, cloning, reporter strain construction, and plasmid and chromosomal DNA purifications were obtained from Sambrook et al. [16]. Enzymes were purchased from either TaKaRa Japan or Promega USA. Two oligonucleotide primers were designed on the basis of the sequences flanking the *nor* genes of *P. aeruginosa* [17]. The *nor* genes of *P. aeruginosa* were amplified by PCR from chromosomal DNA using the primer combinations NorEF (GTAGAATTCCTGGTCTACGTCCTGCAATGAG) and NorHR (GTGAAGCTTCCGATGAGGAACACCACCC). The amplified fragments were subsequently cloned into pMD18-T (TaKaRa) and sequenced. To avoid errors introduced by PCR, the DNA inserts from three individual clones were sequenced and compared. The *nor* genes consensus sequences from *P. aeruginosa* were compared to the nitric oxide reductase genes available in the GenBank database. For construction of the gene knockout mutants, a SacB-based strategy was employed [18]. The PCR product of the *nor*

Table 1
List of strains and plasmids used in this study.

Strain or plasmid	Characteristic(s)[a]	Source or reference
Strains		
Escherichia coli		
DH5α	F- mcrA Δ(mrr-hsdRMS-mcrBC), Φ 80 lacZ ΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara,leu) 7697 galU galK λ-rpsL nupG tonA	Invitrogen
Pseudomonas aeruginosa		
PAO1	Wild type, lab strain	This study
PAN1	*nor* insertion mutant, GmR	This study
PA515	Wild type, clinical strain	Clinic
PAN515	*nor* insertion mutant, GmR	This study
PA196	Wild type, clinical strain	Clinic
PA554	Wild type, clinical strain	Clinic
PA914	Wild type, clinical strain	Clinic
Plasmids		
pEX18Amp	$oriT^+ sacB^+$ gene replacement vector with multiple-cloning site from pUC18, AmpR	[18]
pHpΩ45	Sm^r/Spc^r gene from the R100.1 plasmid, transcription-termination sequences from pMJK4-18 plasmid, SmR/SpcR	[28]
pZ1918Gm	Source plasmid of Gm^r cassette, GmR	[29]
pRK2013	Broad-host-range helper vector, KanR	[19]
pEXB	pEX18Amp containing a *nor* fragment, AmpR	This study
pEXB1lacZ	pEXB1 containing Gm^r-lacZ fragment from pZ1918Gm insert in the *SphI* site, AmpR/GmR	This study

[a] Antibiotic resistance markers: AmpR ampicillin, GmR gentamicin, KanR kanamycin.

Download English Version:

https://daneshyari.com/en/article/1922871

Download Persian Version:

https://daneshyari.com/article/1922871

Daneshyari.com